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Response of the Cultivated Tomato and Its Wild Salt-tolerant Relative *Lycopersicon Pennellii* to Salt-dependent Oxidative Stress: Increased Activities of Antioxidant Enzymes in Root Plastids

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Root plastids of the cultivated tomato *Lycopersicon esculentum* (Lem) exhibited salt-induced oxidative stress as indicated by the increased H_2O_2 and lipid peroxidation levels which were accompanied with increased contents of the oxidized forms of ascorbate and glutathione. In contrast, H_2O_2 level decreased, lipid peroxidation level slightly decreased and the levels of the reduced forms of ascorbate and glutathione increased in plastids of *L. pennellii* (Lpa) species in response to salinity. This better protection of Lpa root plastids from salt-induced oxidative stress was correlated with increased activities of superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidases (POD), monodehydroascorbate reductase (MDHAR), glutathione peroxidase (GPX), glutathione-*S*-transferase (GST) and phospholipid hydroperoxide glutathione peroxidase (PHGPX). In the plastids of both species, activities of SOD, APX, and POD could be resolved into several isozymes. In Lem plastids two Cu/ZnSOD isozymes were found whereas in Lpa an additional FeSOD type could also be detected. In response to salinity, activities of selected SOD, APX, and POD isozymes were increased in Lpa, while in Lem plastids the activities of most of SOD and POD isozymes decreased. Taken together, it is suggested that plastids play an important role in the adaptation of Lpa roots to salinity.

Keywords: Oxidative stress; Roots; Plastids; Tomato; Salt stress; Antioxidative system

Abbreviations: APX, ascorbate peroxidase; ASC, reduced form of ascorbate; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GSH, reduced form of glutathione; GSSG, oxidized form of glutathione; GST, glutathione *S*-transferase; LOX, lipoxygenase; MDA, malondialdehyde; MDHAR, monodehydroascorbate reductase; PHGPX, phospholipid hydroperoxide glutathione peroxidase; POD, guaiacol peroxidase; SOD, superoxide dismutase

INTRODUCTION

The root antioxidant systems have attracted little attention as compared with that of the aerial plant parts in spite of the fact that the roots are also exposed to biotic and abiotic stresses known to induce oxidative stress. Moreover, in the few cases where the responses of root antioxidative systems to various stresses such as salinity,[1–3] heavy metals[4] and chilling[5] were studied, their organellar antioxidative system(s) were overlooked. Nevertheless, the results of such studies suggest that, like the leaves, roots are capable of responding to stress by adjusting their antioxidative capacity. Root cells contain non-photosynthetic plastids, which are responsible for biosynthesis of starch and fatty acids and for nitrogen assimilation.[6] Whether such diverse metabolic activity is associated with active oxygen species (AOS) production and eventually their quenching has awaited clarification.

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The overproduction of AOS is a feature commonly observed under various stress conditions when the equilibrium between formation and detoxification of AOS can no longer be maintained.[7] Plants are equipped with an array of nonenzymic scavengers and antioxidant enzymes that act in concert to alleviate cellular damage under oxidative conditions. This array is composed of low molecular mass antioxidants, such as ASC and GSH, and enzymes that protect against oxidative damage.[8] Several enzymes, each of which exists as multiform isozymes, are involved in the detoxification of AOS. Superoxide dismutase (SOD), which catalyzes the dismutation of superoxide to H_2O_2, plays a key role in AOS detoxification.[9] In plants, hydrogen peroxide scavenging is accomplished by different types of peroxidases or catalase.[10] Ascorbate peroxidase (APX) which catalyzes the first step of the ascorbate–glutathione cycle[11] is the most important peroxidase in H_2O_2 detoxification.[12] Glutathione-dependent peroxidase (GPX) is also involved in the scavenging of soluble hydroperoxides.[13]

Peroxidation of membrane lipids, which render the lipid bilayer leaky,[14] can occur nonenzymically by AOS[15] or enzymically by lipoxygenase (LOX).[16] Detoxification of membrane lipid hydroperoxides can be maintained by the activities of enzymes such as phospholipid hydroperoxide glutathione peroxidase (PHGPX) and glutathione-*S*-transferase (GST). PHGPX is involved in scavenging of phospholipid hydroperoxides[17] and GST activity represents another class of antioxidant enzymes, which is also responsible for detoxification of cytotoxic lipid peroxidation products, such as 4-hydroxyalkenals.[18] Recently,[3] we have shown in root homogenate prepared from salt-stressed *L. esculentum* (Lem), a salt-sensitive species, decreased activities of SOD, catalase (CAT) and APX as well as decreased contents of the reduced forms of ascorbate and glutathione. In contrast, increase in the activities of these enzymes and increased contents of the reduced forms of ascorbate and glutathione were found in the roots homogenates of salt-stressed *L. pennellii* (Lpa), a salt-tolerant species and a wild relative of Lem. These contrasting salt-induced responses were correlated with alleviation of salt-dependent oxidative damage in Lpa roots and increased salt-dependent oxidative damage in those of Lem.

In the present work, the effect of salt-induced oxidative stress was investigated for the first time in plastids isolated from salt-stressed plants. We show that these organelles are vulnerable to oxidative stress, in that they produce H_2O_2 and peroxidation of their lipids occurs. We also show that root plastids possess at least two lines of enzymic defense against salt-induced oxidative damage. Moreover, several isozymes of SOD, POD, and APX were revealed in this organelle and those isozymes that were affected by salinity were identified.

MATERIAL AND METHODS

Plant Material

Plants of the cultivated tomato, *Lycopersicon esculentum* Mill. cv. M82 (Lem) and its wild salt-tolerant relative *L. pennellii* (Corr.) D'Arcy acc. Atico (Lpa) were grown hydroponically in a greenhouse as described earlier.[19] Salt treatment started at the stage of about four true leaves by increasing the NaCl concentration by 25 mM per day to a final concentration of 100 mM. Roots were harvested 14 days after the completion of salt treatment.

Purification of Plastids

Roots (20 g) were chopped in a Waring blender (5 ml/gFW) in homogenization buffer [50 mM Hepes (7.5), 2 mM EDTA, 5 mM ascorbate, 0.33 M sorbitol]. The plastids were sedimented (1000*g*, 5 min) and resuspended in the same buffer and loaded on 20% Percoll cushion.[20] The pelleted plastids were collected and diluted in the same buffer. The cross-contamination of plastids by mitochondria and peroxisomes was 3% (using cytochrome C oxidase as a marker) and 11% (catalase as a marker), respectively.[19] These values are well in the range described by other authors for cross-contamination of chloroplasts, mitochondria, and peroxisomes.[21–23] For measurement of membrane lipid peroxidation, plastids were resupended in sorbitol-omitted buffer. Membranes were precipitated by centrifugation (150,000*g*, 30 min). All operations were conducted at 4°C.

Enzyme Assays

Total SOD, APX, monodehydroascorbate reductase (MDHAR; EC 1.6.5.4), dehydroascorbate reductase (DHAR; EC 1.8.5.1), glutathione reductase (GR; EC 1.6.4.2) activities were assayed as described previously.[19] The activities of Cu/Zn-and FeSODs were measured using 3 mM KCN or 5 mM H_2O_2 as inhibitors, respectively.[24] Total LOX (EC 1.13.11.12) activity was assayed using linoleic acid as substrate, following.[25] GST (EC 2.5.1.18) and GPX (EC 1.11.1.9) activities were determined according to Ref. [13] using 1-chloro, 2,4-dinitrobenzene and H_2O_2 as substrates, respectively. In order to minimize possible interference of POD with the GPX assay, the pH of the reaction medium was buffered at 7.0, as compared with the pH

optimum of POD which was shown to be in the range of 4.5–5.0.[26] PHGPX (EC 1.11.1.12) activity was determined using phosphatidylcholine hydroperoxide as a substrate.[27]

Isozyme Analysis

Aliquots of protein (50 μg per lane) were loaded on native PAGE.[28] Activities of POD, APX, and SOD isozymes were detected according to Refs. [29–31], respectively. Protein was assayed according to Ref. [32].

Other Analytical Methods

Lipid peroxidation of membranes isolated from osmotically-shocked plastids was determined as the amount of malondialdehyde (MDA, $\varepsilon = 155\,mM^{-1}\,cm^{-1}$), a secondary product of lipid peroxidation, according to Ref. [33]. H_2O_2 content was assayed according to Ref. [34]. This assay, known as FOX1 is widely used for the quantification of water-soluble peroxides (e.g. H_2O_2) in animal and plant specimens and is based on ferrous ion oxidation in the presence of the ferric ion indicator xylenol orange. For the determination of total ascorbate and ASC contents,[35] aliquots of plastids were divided into equal parts. Total ascorbate (ASC + DHA) was determined after reducing the DHA to ASC using dithiothreitol. DHA content was then calculated from the difference between total ascorbate and ASC. Reduced and total glutathione were determined according Ref. [36].

RESULTS

Plastidic H_2O_2 level increased (160%) in salt-treated Lem while it decreased (50%) in salt-treated Lpa (Fig. 1A). Under the same conditions, plastidic membrane lipid peroxidation increased (130%) in Lem and slightly decreased in Lpa (Fig. 1B). Determination of GST and PHGPX activities which are associated with detoxification of membrane lipid hydroperoxides, revealed a slight decrease in plastids of salt-treated Lem plants and increase (150 and 180%, respectively) in those of the salt-treated Lpa (Fig. 2). In contrast, the activity of LOX, which catalyzes the hydroperoxidation of membrane lipids, increased (130%) in Lem and was virtually unaffected by the salt treatment in Lpa plastids (Fig. 2).

The capacity of the plastids to quench AOS under salt treatment, as indicated by the activities of SOD, APX, GPX, and POD, is shown in Fig. 3 These

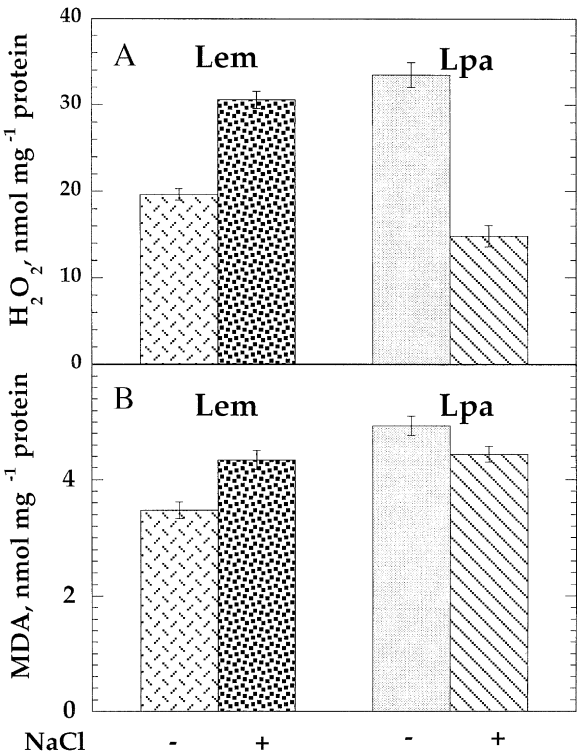

FIGURE 1 Effect of salt treatment on hydrogen peroxide (A) and MDA (B) contents in root plastids of the cultivated tomato (Lem) and the wild species (Lpa). Plastids were isolated from plants grown for 14 days under 0 (control) or 100 mM NaCl. Values represent the means ± SE of three independent experiments.

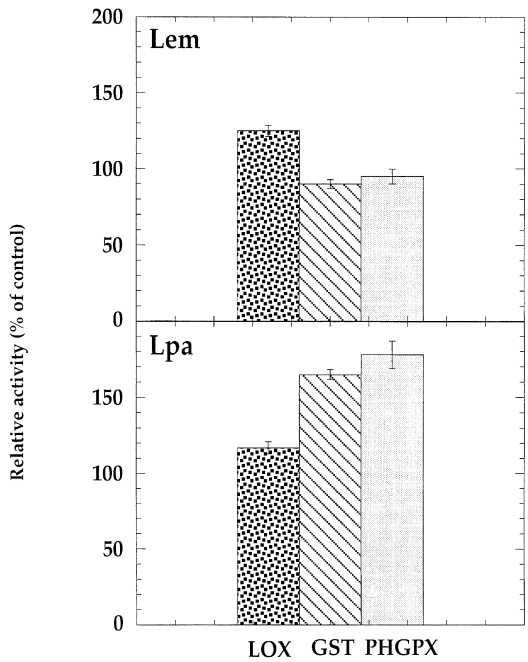

FIGURE 2 Effect of salt treatment on the activities of LOX, GST and PHGPX in root plastids of the cultivated tomato (Lem) and the wild species (Lpa). Enzyme activities in plastids isolated from control plants were: Lem–LOX, 0.05 ± 0.003; GST, 0.10 ± 0.006; PHGPX, 0.04 ± 0.003 (μmol min^{-1} mg^{-1} protein). Lpa–LOX, 0.06 ± 0.004; GST, 0.16 ± 0.015; PHGPX, 0.05 ± 0.004 (μmol min^{-1} mg^{-1} protein). Plastids were isolated from plants grown for 14 days under 0 (control) or 100 mM NaCl. The activity of each enzyme was expressed relative to its corresponding activity in the respective non-salinized control. The results are the means of three independent experiments ± SE.

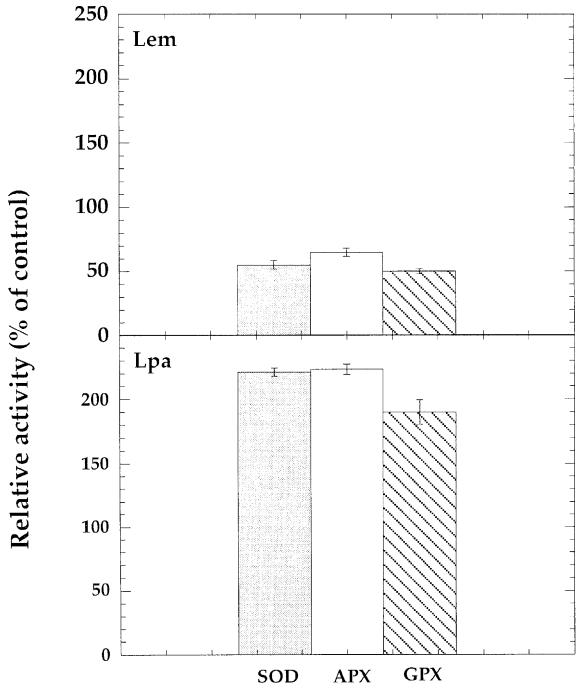

FIGURE 3 Effect of salt treatment on the activities of SOD, APX, GPX and POD in root plastids of the cultivated tomato (Lem) and the wild species (Lpa). Enzyme activities in plastids isolated from control plants were: Lem–SOD, 1.20 ± 0.1 (Units $min^{-1} mg^{-1}$ protein); APX, 1.52 ± 0.17; GPX, 0.43 ± 0.02; POD, 0.22 ± 0.01 (μmol $min^{-1} mg^{-1}$ protein). Lpa–SOD, 3.30 ± 0.46 (Units mg^{-1} protein min^{-1}); APX, 1.34 ± 0.08; GPX, 0.45 ± 0.03; POD, 0.29 ± 0.01 (μmol $min^{-1} mg^{-1}$ protein). Additional details as in Fig. 2.

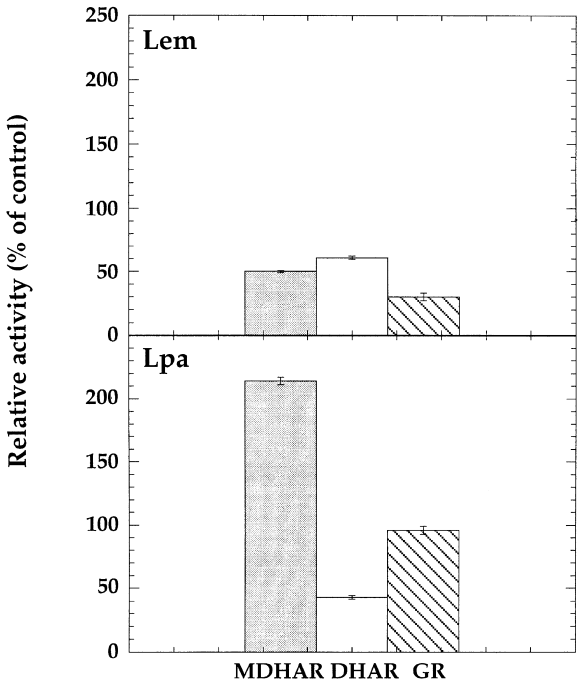

FIGURE 4 Effect of salt treatment on the activities of MDHAR, DHAR and GR in root plastids of the cultivated tomato (Lem) and the wild species (Lpa). Enzyme activities in plastids isolated from control plants were: Lem–MDHAR, 1.02 ± 0.14; DHAR, 0.23 ± 0.01; GR, 0.20 ± 0.01 (μmol $min^{-1} mg^{-1}$ protein). Lpa–MDHAR, 1.41 ± 0.17; DHAR, 0.16 ± 0.04; GR, 0.05 ± 0.005 (μmol $min^{-1} mg^{-1}$ protein). Additional details as in Fig. 2.

activities decreased (about 40–50%) in Lem and increased (170–230%) in Lpa. The activities of the ascorbate- and glutathione-regenerating enzymes, MDHAR, DHAR, and GR, decreased by about 50% in the plastids of salt-treated Lem. In plastids of salt-treated Lpa MDHAR activity increased (220%), that of GR did not change and that of DHAR was reduced (Fig. 4).

The possibility that some isozymes of the antioxidant enzymes are differentially affected by salt treatment was addressed. Two forms of Cu/ZnSOD were identified in Lem plastids, of which LemCu/ZnSOD2 was down-regulated by the salt treatment (Fig. 5A). In Lpa plastids, salt treatment resulted in an increased activity of LpaCu/ZnSOD1 and the appearance of LpaCu/ZnSOD2, which was below detection level in control plastids (Fig. 5A). In addition, unlike in Lem, FeSOD activity was detected only in Lpa plastids (Fig. 5A).

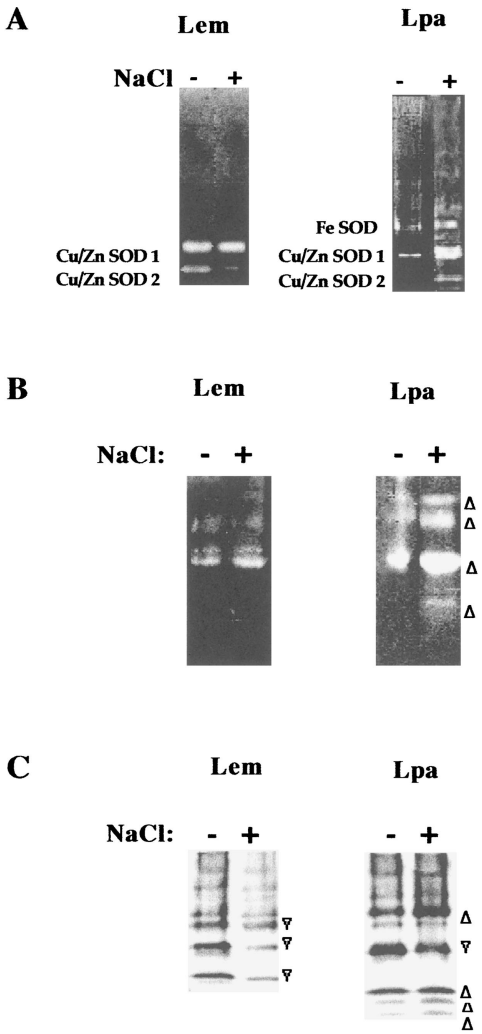

FIGURE 5 Identification of SOD (A), APX (B) and POD (C) isozymes in root plastids of the cultivated tomato (Lem) and the wild species (Lpa). Plastids were isolated from plants grown for 14 days under 0 (control) or 100 mM NaCl. Aliquots of 50 μg protein were loaded and separated on a non-denaturing polyacrylamide gel and stained for SOD, APX and POD activities, respectively.

TABLE I Ascorbate and glutathione content in plastids isolated from roots of the cultivated tomato (Lem) and the wild species (Lpa), grown for 14 days under 0 or 100 mM NaCl. Values are expressed as the means ± SE of three different experiments. Significance of difference between means was estimated using the Student *t*-test

Species	NaCl (mM)	ASC (nmol mg^{-1} protein)	DHASC (nmol mg^{-1} protein)	ASC/DHASC	GSH (nmol mg^{-1} protein)	GSSG (nmol mg^{-1} protein)	GSH/GSSG
Lem							
	0	106.90 ± 4.27	68.53 ± 4.60	1.60 ± 0.08	2.52 ± 0.10	0.14 ± 0.003	17.21 ± 1.73
	100	79.80 ± 5.67*	116.25 ± 8.10†	0.70 ± 0.03†	2.10 ± 0.10*	0.25 ± 0.003†	8.50 ± 0.71†
Lpa							
	0	37.20 ± 2.01	56.03 ± 2.73	0.60 ± 0.05	7.60 ± 0.27	0.28 ± 0.003	35.30 ± 1.23
	100	46.91 ± 1.90*	22.70 ± 2.10†	2.20 ± 0.07†	20.58 ± 1.11†	0.31 ± 0.003‡	65.40 ± 4.00†

* Differences from control values were significant at $P < 0.05$.
† Differences from control values were significant at $P < 0.001$.
‡ Differences from control values were significant at $P < 0.01$.

When compared on the same gel LemCu/ZnSOD2 and LpaCu/ZnSOD1 had the same electrophoretic mobility while LemCu/ZnSOD1 and LpaCu/ZnSOD2 exhibited different mobilities (data not shown). Two APX isozymes were noticeable on native gels of Lem plastids (Fig. 5B). Salt treatment did not affect the activities of these two bands. In Lpa plastids salinity increased the activity of three APX isozymes, and a fourth isozyme, which could not be detected under control conditions, became visible under these conditions (Fig. 5B).

Staining plastidic peroxidases, using *o*-dianisdine as an electron donor, revealed that under normal growth conditions each of the two tomato species possess a set of peroxidase isozymes (Fig. 5C). Some of Lem and Lpa isozymes shared similar electrophoretic mobilities while other exhibit different mobilities. In Lem plastids, salt treatment resulted with a general decrease in all POD isozymes. In Lpa plastids, the activities of four POD isozymes were increased (marked with an upward pointed arrow head) and that of one decreased (marked with a downward pointed arrow head) in response to salt treatment (Fig. 5C).

Inherent ASC and GSH contents varied between the two tomato species. ASC content was 290% higher in Lem plastids as compared with those of Lpa while GSH content was 300% higher in the latter plastids as compared with that of Lem (Table I). Moreover, in response to salt treatment ASC content decreased (27%) and DHA content increased (150%) in Lem plastids. In contrast, ASC content increased (150%) and that of DHA decreased (55%) in Lpa plastids under these conditions. Consequently, ASC/DHA ratio decreased (57%) in plastids of salt-treated Lem and it increased (370%) in those of treated Lpa.

In response to salt treatment GSH content of Lpa plastids increased (270%) and was accompanied with a modest increase in GSSG content. These changes resulted with increased (200%) GSH/GSSG ratio (Table I). In plastids of salt-treated Lem GSH level was slightly decreased while that of GSSG increased (180%). Consequently, a 50% decrease in GSH/GSSG ratio was found in these plastids (Table I).

DISCUSSION

As in the case of whole root,[3] a salt-induced oxidative stress was apparent in root plastids of the cultivated species Lem. This was indicated by the increased levels of H_2O_2 and lipid peroxidation after 14 days of salinization (Fig. 1). Furthermore, the increase in the levels of these oxidative-stress indicators was accompanied by decreased activities of most of the plastidic antioxidant enzymes

(Figs. 2–4). It should be noted that determination of MDA, a breakdown product of lipid as a result of peroxidation, is rather an indirect method to evaluate the stress-induced damage caused to membrane lipids.[37] For this reason malondialdehyde content is referred here as an indicator for lipid peroxidation and not as a quantitative measure of the latter. In contrast, in plastids of salt-treated Lpa plants the level of H_2O_2 decreased and that of lipid peroxidation was not changed (Fig. 1). These responses were accompanied by increased activity of a set of antioxidant enzymes (Figs. 2–4). Mittova *et al.*[19] estimated that the inherent activities of SOD and the ascorbate–glutathione enzymes in root plastids comprise between 20 and 50% of their activities in the root extract. The observation that the responses of the plastidic antioxidative system of both species to salinity were qualitatively similar to those described by us recently in root extracts[3] suggests that the former system significantly contributes to the antioxidative capacity of the whole root also under salinity.

The decreased H_2O_2 level in plastids of salt-treated Lpa roots cannot be explained solely by increased activities of the H_2O_2 quenchers APX, GPX, and POD (Fig. 3) since these changes were counteracted by a similar increase in SOD activity (Fig. 3). This decrease, however, may be explained by the involvement of ASC in nonenzymic H_2O_2 detoxification as increased level of ASC was observed in plastids of salt-treated Lpa roots (Table I). Furthermore, this higher level of reduced ascorbate was accompanied by a decreased level of DHA (Table I) and an increased MDHAR activity (Fig. 4), indicating a higher capacity for ASC regeneration under these conditions. It is possible, however, that part of the decrease in total ascorbate content (Table I) was caused by enhanced catabolism of either the reduced or oxidized ascorbate forms. Further support for the role of ASC in H_2O_2 detoxification can be drawn from the observation that ASC content decreased and DHA content increased in plastids of salt-stressed Lem roots (Table I). It could be argued that the observed changes in H_2O_2 content resulted from interference of ASC with the FOX1 determination rather than from a genuine change. This question was recently addressed by Bleau *et al.*[38] who showed that in the presence of 1.25 μM ASC, an overestimation of about 20–30% in H_2O_2 concentration was obtained. In our experiments ASC concentrations were in the range of 0.5–1.0 μM, therefore, the overestimation of H_2O_2 content is expected to be smaller than 20%. We found that under salt stress conditions, the plastidic ASC content increased significantly (150%) in the salt-tolerant and decreased (27%) in the salt-sensitive species. Simultaneously, in the same plastids, the H_2O_2 content (as determined by the FOX1 assay) decreased (50%) in the salt-tolerant and increased (160%) in the salt sensitive species. Therefore, the change in H_2O_2 we observed would have been even larger if corrected for the ASC effect and cannot be explained as an artifact.

The massive GSH content increase (270%) in plastids of salt-treated Lpa plants (Table I), can be explained as the result of increased GSH synthesis.[39] This conclusion is supported by the observations that: (i) γ-glutamylcysteine synthetase activity increased in plastids of salt-treated Lpa roots (Mittova *et al.*, data not shown), (ii) the pool size of GSSG, as compared to that of GSH, was far too low to support the observed GSH increase via its regeneration (Table I), (iii) GR activity did not change under salinity and thus could not support increased demand for GSH regeneration (Fig. 4). However, this suggestion cannot be ascribed to plastids of salt-stressed Lem species where their GSH content decreased (Table I) and the activity of γ-glutamylcysteine synthetase activity remained as in control (Mittova *et al.* data not shown).

APX activity in root plastids (see legend of Fig. 3) was comparable (on per protein basis) to that of leaf chloroplasts (Mittova *et al.*, data not shown). However, in both species POD activity in root plastids was much higher as compared with its activity in the chloroplasts (plastidic and chloroplastic APX/POD were of about 5 and 100, respectively). This high inherent plastidic POD activity may reflect its relative importance in H_2O_2 detoxification in the roots of both species. The salt-dependent increase in POD activity, which occurred only in Lpa plastids (Fig. 3) may contribute, at least in part, to the higher capacity of this species to detoxify H_2O_2 (Fig. 1). POD activity, however, may also play a role in the oxidation of phenolic metabolites in the root plastids. Accumulation of such phenolic compounds has been observed under various stresses.[40]

Lipid hydroperoxides are the products of unsaturated fatty acids oxygenation. Oxygenation of fatty acids may be catalyzed enzymically by LOX or may be produced nonenzymically by AOS.[16] Lipid hydroperoxides level is also determined by the activities of GST and PHGPX. GST is known mainly as xenobiotics detoxifier and was also shown to alleviate membrane lipid peroxidation,[41] while PHGPX, a member of the glutathione peroxidase enzyme family, reduces phospholipid hydroperoxides.[17] PHGPX was shown as the only glutathione peroxidase to be induced in tomato by mechanical stimulation,[42] suggesting a significant role of this enzyme in the antioxidant defense in tomato. It is likely that the increased lipid peroxidation content in plastids of salt-treated Lem plants (Fig. 1) resulted from both increased peroxidation rate and decreased capacity to scavenge lipid peroxidation products.

This is supported by the findings that in these organelles both H_2O_2 level and LOX activity increased (Figs. 1 and 2, respectively) while GST and PHGPX activities slightly decreased (Fig. 2). In contrast, the decreased lipid peroxidation level in plastids of salt-treated Lpa plants, as compared to their respective control, can be explained, at least in part, by the decreased H_2O_2 content and the increased GST and PHGPX activities (Figs. 1 and 2). Gossett *et al.*[43] suggested that GST activity has a role in alleviation of salt stress in cotton calli. This was based on the observations that in response to salinity the level of lipid peroxidation of salt-sensitive cotton calli increased and the activity of GST remained as in the control while in the salt-tolerant calli lipid peroxidation remained as in the control and GST activity increased. Further support for the involvement of GST in alleviation of salt stress was brought by Ref. [44] who showed increased salt tolerance in transgenic tobacco seedlings overexpressing GST cDNA.

Isozymes of antioxidant enzymes were shown to be present in cytosol,[45] chloroplasts,[46] mitochondria and peroxisomes[21] of plant cells. Here we provide evidence for the presence of several isozymes of SOD, APX, and POD in root plastids and that their expression is differentially affected by salinity. Salinization resulted with increased activity of two Cu/ZnSOD isozymes (Fig. 5A), in particular that of Cu/ZnSOD1 which its activity was found in the supernatant of osmotically-disrupted plastids (Mittova *et al.*, data not shown). FeSOD activity could be detected on a non-denaturing polyacrylamide gel only in the plastids of Lpa, suggesting an important role for this isozyme in the plastidic antioxidant system of this species (Fig. 5A). This was confirmed also by differential inhibition as described by Ref. [24] (Mittova *et al.*, data not shown). A comparative study of different SOD types in other root cell organelles (mitochondria and peroxisomes) indicated that FeSOD activity resides exclusively in the plastids of the salt-tolerant species, this excludes an artifact due to cross-contamination (Mittova *et al.*, data not shown). However, the finding that in Lpa plastids FeSOD activity only slightly increased in response to salt treatment (Fig. 5A) is puzzling because in the chloroplasts of salt-treated Lpa plants FeSOD activity increased (200%) (Mittova *et al.*, data not shown). FeSOD has been shown to be less sensitive than Cu/ZnSOD to inhibition by H_2O_2.[47] It is possible that the inherent FeSOD activity found in Lpa plastids (Fig. 5A) enables these organelles to cope with the salt-dependent buildup of AOS before upregulation of the antioxidative system commenced.

In agreement with the effect of salinity on total APX activity (Fig. 3) the activities of all detectable APX isoforms were also increased in Lpa plastids (Fig. 5B). In contrast, total APX activity in plastids of salt-stressed Lem decreased (Fig. 3). This is, however, could not be confirmed by the APX activity gel (Fig. 5B). This discrepancy awaits for further clarification. Activities of four Lpa POD isozymes increased and of one isozyme were decreased in response to salinity (Fig. 5C). This implies that, unlike APX isozymes, only part of the plastidic POD isozymes may have a role in the adaptation of this species to salt stress, however, the significance of this observation is not known at this stage.

Acknowledgments

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